

NEURAL CONTROL OF RADULAR RETRACTOR
MUSCLES OF THE PULMONATE SNAIL,
PLANORBARIUS CORNEUS (L.): FUNCTIONAL
ANATOMY AND PROPERTIES OF NEUROMUSCULAR
UNITS

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SUMMARY

The paired supralateral radular retractor muscles of *Planorbarius* are innervated by eight (four pairs) of buccal motoneurons. Each motoneurone supplies both muscles, the ipsi- and contralateral motor responses being similar, and identical to those produced by its contralateral partner. This arrangement apparently secures equivalence of motor output to the two muscles, irrespective of discrepancies in spiking between homologous motoneurons across the midline, and relates to the strong mechanical linkage between the muscles exerted by the radula. The neuromuscular units are essentially non-overlapping, have properties which range from tonic to phasic, and may act independently providing the flexibility in control required of bifunctional muscles participating in both tensing and retraction of the radula during each feeding cycle.

INTRODUCTION

The feeding system of gastropod molluscs is useful for investigating the generation of relatively stereotyped, cyclic, motor sequences (see Kandel, 1976, 1979). The buccal mass and ganglia form a discrete system which can be isolated from the animal without apparently altering normal motor control. In more intact preparations studies can be made of higher-order control (Davis, Siegler & Mpitsos, 1973; Davis *et al.* 1974; Gillette, Kovac & Davis, 1978), the effects of motivational variables (Kupfermann, 1974; Lee & Palovcik, 1976; Sussewein, Kupfermann & Weiss, 1976), and of the position of feeding within behavioural hierarchy of the animal (Davis, Mpitsos & Pinneo, 1974; Davis *et al.* 1977).

From these investigations a broad picture of the neural circuitry within the buccal ganglia has emerged. In general there is clear separation between circuits controlling odontophoral protraction and retraction which are largely mutually antagonistic. The generation of rhythmic activity, at least in *Helisoma*, appears to involve the intrinsic properties of groups of electrically coupled interneurons (Kater, 1974; Merickel, Eyman & Kater, 1977; Kaneko, Merickel & Kater, 1978).

Rather less attention has been paid to the control of any one pair or set of buccal muscles, though recently Cohen, Weiss & Kupferman (1978) have described some aspects of peripheral integration underlying the control of the accessory radular closer muscles in *Aplysia*.

Here and in a companion paper (R. C. Brace & D. L. J. Quicke, in preparation) we describe the innervation of the pair of supralateral radular retractor (SLR) muscles (Hembrow, 1973) of the freshwater pulmonate mollusc, *Planorbarius corneus* (L.). Attention was focused on these muscles since they have a dual role, serving to both tense and retract the radula. This neuromuscular system thus affords the opportunity of studying integrative mechanisms leading to flexibility in motor output.

To demonstrate the multifunctional role of the SLR muscles we first describe their functional anatomy. Then, by reference to motor events occurring during the feeding cycle we reconstruct the likely temporal sequences of activity in the various regions of the muscles which, in turn, directly reflects the varying participation of a number of neuromuscular units. The properties of these units are considered together with coupling between them at the motoneuronal level. We also describe the morphology of the SLR motoneurons. The significance of all cells providing bilateral innervation is discussed.

MATERIALS AND METHODS

Materials

Animals originating from N. Wales were maintained in 10 l tanks and were fed on lettuce and porridge oats. Most animals bred in captivity and survived for over 1 year. Animals of 2–3 cm shell diameter were selected for experimentation. These were both of the red type in which melanin is lacking and of the dark variety. The saline used was based on that described by Berry (1972) and had the following composition: NaCl, 34.0 mM; KCl, 2.0 mM; MgCl₂.6H₂O, 2.0 mM; CaCl₂.6H₂O, 4.5 mM; NaHCO₃, 10.0 mM; NaH₂PO₄.2H₂O, 0.4 mM; pH 7.5.

Dissection

After removal of the shell, mantle and viscera, an incision was made through the dorsal body wall in order to expose the buccal mass. The oesophagus was pulled anteriorly through the nerve ring and pinned so as to incline the longitudinal axis of the buccal mass (which in the resting state runs topographically vertical) forwards, thereby facilitating access to the buccal ganglia. Animals at this stage of dissection constituted our *semi-intact preparations*.

Further dissection consisted of removing the buccal mass from the headfoot. When the circumoesophageal nerve ring was required, its emergent nerves (with the exception of the cerebrobuccal connectives) were cut, thus allowing it to be removed simultaneously. These dissections provided our *intact buccal mass preparations*.

To fully observe contractions in all regions of the SLR muscles we found it necessary to make sagittal cuts through the ventral wall of the buccal mass and the odontophoral cartilage. Consequently the retractor muscles could be displayed by drawing the two ventral halves of the buccal mass apart laterally. Finally, the suprmedian

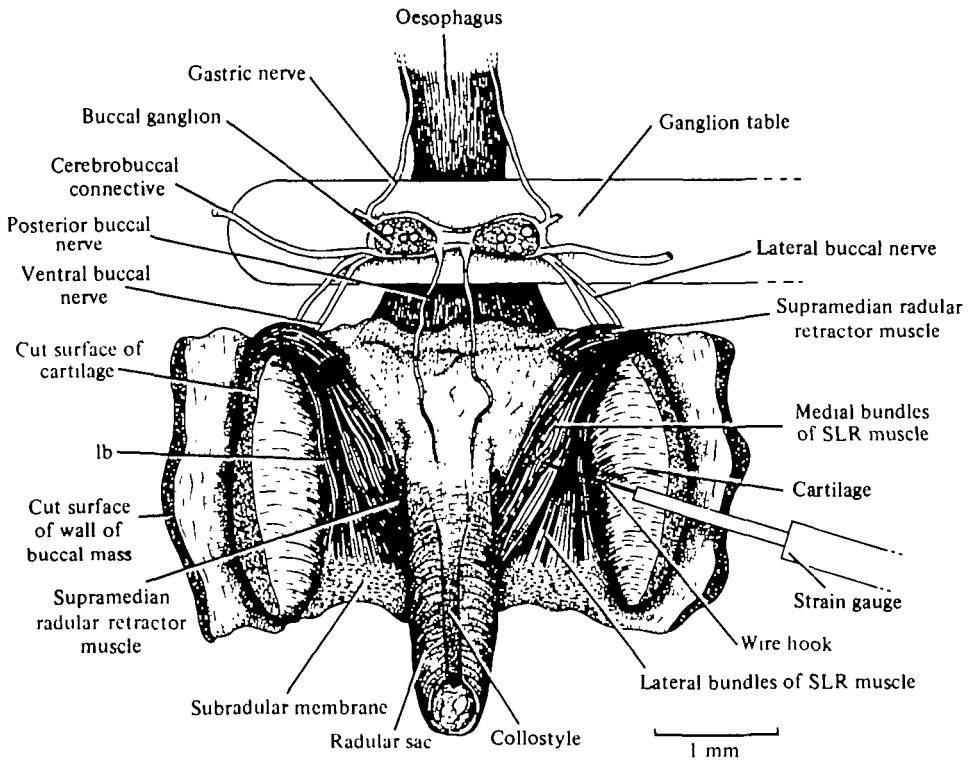


Fig. 1. Dissected buccal mass preparation of *Planorbarius* and accompanying buccal ganglia which are stabilized by a micro-platform. Note the functional division of the SLR muscles into lateral fibres which insert on the subradular and buccal epithelia, and medial bundles of fibres which largely insert on the radular sac.

radular retractor muscles (Carriker, 1946a; Hembrow, 1973) which lie ventral to the SLR muscles were cut, and the radular sac separated from adjacent tissues, turned anteriorly and secured in position. This dissection stage provided our *dissected buccal mass preparations* (Fig. 1).

In all preparations the buccal ganglia were stabilized on a wax-coated micro-platform which permitted stable intracellular recording whilst the buccal mass underwent feeding movements. Its insertion between the ganglia and buccal mass was made easier by first severing the dorsal buccal nerves, trimming away the salivary glands, and by carefully freeing the proximal length of each gastric nerve from the oesophagus. The ganglia were secured to their support by pins passing through adjacent connective tissue.

Physiological techniques

Conventional recording methods and equipment were used to monitor activity and pass current into buccal neurones. The muscle contractions produced by SLR motoneurone stimulation were monitored using a Grass isotonic strain gauge and

displayed, together with motoneuronal activity, on a Grass Polygraph pen recorder. To measure changes in tension, a small hook (fashioned from a fine pin and attached to the strain gauge) was inserted under a bundle of fibres whilst one or more SLR motoneurons were depolarized. Although this method displayed well the temporal aspects of contraction it was not a reliable measure of the absolute magnitudes of tensions produced by the various muscle bundles as we were unable to firmly stabilize their frail insertion sites.

Neural anatomy

To visualize neuronal profiles we injected nickel ions into cell somata and, after at least 12 h incubation at 10 °C, subsequently added rubeanic acid (Quicke & Brace, 1979). These preparations were then intensified (see Tyrer & Bell, 1974) using Bacon & Altman's (1977) modification for whole-mounts. We have found that rubeanate complexes intensify as successfully as do sulphides (D. L. J. Quicke, R. C. Brace & P. Kirby, in preparation).

RESULTS

Innervation of the SLR muscles

In addition to the cerebrobuccal connective, each buccal ganglion gives off four nerves to the buccal mass, oesophagus and stomach (Figs. 1, 2, 4). The SLR muscles are supplied by the lateral and ventral routes and possibly by the posterior buccal nerves.

Functional anatomy of the supralateral radular retractor (SLR) muscles

These muscles, which are the bulkiest in the feeding apparatus, run anteriorly (Figs. 1, 2) from origins on the lateral and ventrolateral margins of the posterior horns of the odontophoral cartilage which is deeply U-shaped in cross-section. The insertions are on (1) the dorsolateral margins of the radular sac, (2) the subradular epithelium, and more laterally (3), to the buccal epithelium. Most bundles of fibres have a curved course (Fig. 2) to their insertions which parallels the surface of the cartilage. However, the most lateral bundles have more or less straight paths towards the subradular membrane and buccal epithelium.

Additionally, on each side of the midline, there is a discrete bundle of fibres (1b, Fig. 1) which, although considered as part of the SLR muscle by Hembrow (1973), is not dealt with here.

Contraction of the medial SLR bundles pulled the radular sac and radula posteriorly (see Fig. 2) and drew the cartilage arms towards the midline. In contrast, shortening of the lateral bundles of fibres tensed the lateral regions of the radula over the cartilage lip.

Based on these observations we have reconstructed the activity patterns (Table 1) in the medial and lateral parts of these muscles during the feeding cycle (Fig. 3). At the end of odontophoral protraction (Fig. 3*a-b*) the cartilage became partially flattened, thereby allowing a maximal area of the radula to contact the substratum during the ensuing rasping phase (Fig. 3*b-c*). During rasping the radula was immobilized over the cartilage by antagonistic activity of the protractor and retractor.

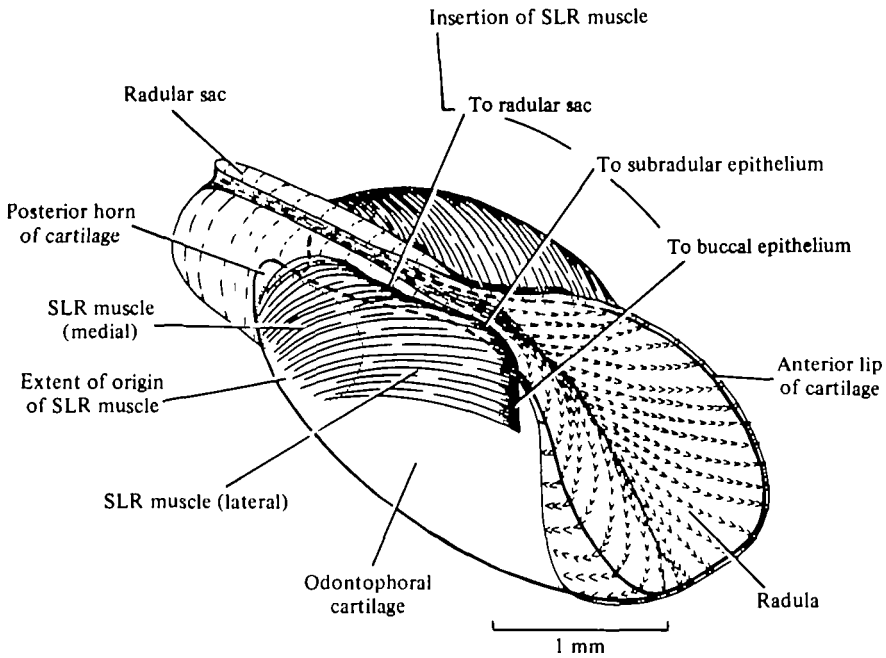


Fig. 2. Diagrammatic anterolateral view from the right of the radular assembly of *Planorbarius* to show the disposition of the SLR muscles. The outline of the odontophoral cartilage is depicted by a thick solid line, except where overlain by the retractor muscles where it is dashed. The line of insertion of the right SLR muscle is heavily shaded. A thin line denotes the extent of the radular membrane which covers the anterior portion of the cartilage. Upon retraction, the radular halves fold towards the midline; contraction of the lateral parts of the retractor muscles, however, pulls the radular halves posterolaterally over the lip of the cartilage, thus spreading and tensing it.

Table 1. Reconstruction of the likely temporal sequences of activity of the lateral and medial fibre bundles of the SLR muscles during the feeding cycle, together with those of other buccal muscles

	Radular protraction	Radular rasp	Radular retraction
SLR muscle (lateral bundles)	Shaded vertical bars	Shaded vertical bars	
SLR muscle (medial bundles)		Shaded vertical bars	Shaded area
Supramedian radular retractor		Shaded vertical bars	Shaded area with a question mark
Radular protractors	Shaded area	Shaded vertical bars	
Intrinsic cartilage tensors		Shaded vertical bars	

Shaded vertical bars denote activity resulting principally in tensing of the radula over the cartilage: overt movements of the radula over its supporting structure are shown by shaded areas.

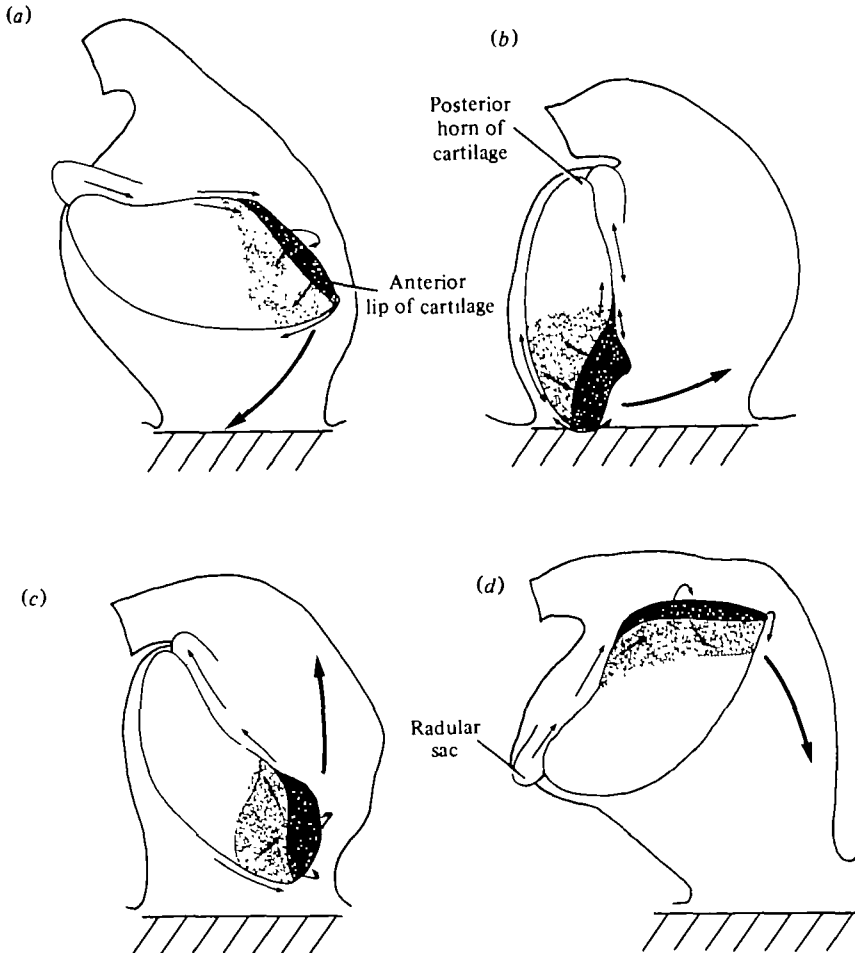


Fig. 3. Diagrams to show movements of the odontophoral cartilage, radula and radular sac during the course of the feeding cycle of *Planorbarius*. Sequence stages illustrated are (a) at rest, (b) at commencement of rasp, (c) start of retraction, following termination of the rasp and (d) end of radular retraction prior to return to the resting position. The subradular membrane is shown stippled, with that part overlying the medial face of the cartilage heavily so. Movements of the odontophore and, secondly, of the radula and radular sac occurring between the stage depicted and the next in sequence, are indicated, respectively, by large and small arrows. Double-headed lines indicate tensing rather than overt movement of the radula over the cartilage lip. Note the distortion of the anterior cartilage lip in (b) due to forces imposed at the working surface.

muscles, the lateral parts of the SLR muscles in particular acting to spread the radula. However, as the odontophore travelled over the substratum the cartilage arms were progressively withdrawn by increasing contraction of the medial SLR bundles. This was followed by radular retraction during which further shortening of the medial components (Fig. 3c-d) was responsible for the rapid retreat of the radula posteriorly over the cartilage lip. Simultaneously the lateral parts of the muscles relaxed. Once fully retracted, the medial regions of the muscles, by virtue of their insertions being firmly held by the converging cartilage arms, also acted to draw the posterior horns of the cartilage together, thus further immobilizing the radular sac.

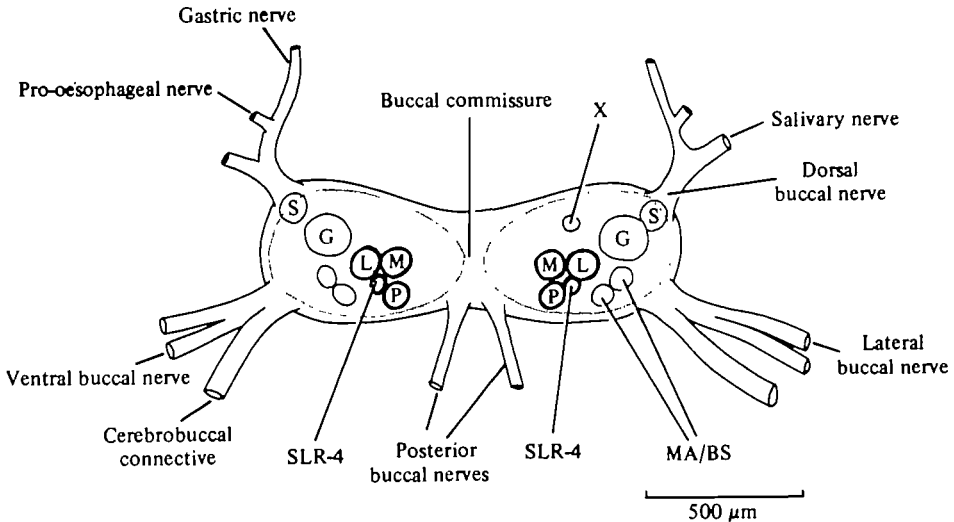


Fig. 4. Diagram of the topographically posteroventral surfaces of the buccal ganglia and emergent nerves of *Planorbarius* to show locations of the four pairs of SLR motoneurons, together with those of a number of other large identified cells. Abbreviations: L, lateral SLR motoneurone; M, medial SLR; P, phasic SLR; SLR-4, 4th SLR; G, gastroesophageal neurone; S, neurone innervating the salivary gland; MA/BS, neurones innervating the mandibular approximator and buccal sphincter muscles; ×, position of cell 4 of Berry (1972).

It must not be assumed, however, that the feeding cycle of *Planorbarius* is completely stereotyped. For example, several rasps sometimes occurred between each major retraction. Secondly, when *Planorbarius* was feeding on larger pieces of food, as opposed to a thin layer, mastication by the jaws became important. In order to bite these items effectively, they were manipulated by the tensed radula which acted as a surface against which the jaws worked; similar behaviour has been noted in *Lymnaea* (Carriker, 1946b).

Identification and anatomy of SLR motoneurons

We searched the buccal ganglia for candidate SLR motoneurons by depolarizing impaled cells thus causing them to spike, and observing any resultant motor action. Eight cells were located which could be reliably identified from animal to animal by their positions within the cell-map and their motor effects. Typical locations are shown in Fig. 4 together with those of a number of other neurones which we have found to be helpful positional indicators. We have labelled the four motoneurons within each ganglion: SLR-L (lateral), SLR-M (medial), SLR-P (phasic) and SLR-4 (4th). The average soma dimension of each of these cells was, respectively, 80, 70, 60 and 30 μm in animals of 2.5 cm shell diameter.

A comparison of our cell-map with that of Berry (1972) showed that cells (G) (innervating the oesophagus and stomach) and (S) (supplying the salivary gland) correspond to Berry's cells 1 and 2, whilst our SLR-L is obviously his cell 3.

In dissected buccal mass preparations, a 1:1 correspondence between action potentials in our candidate motoneurons and SLR muscle twitches was evident, even at high frequency. These cells were found to innervate both ipsi- and contra-

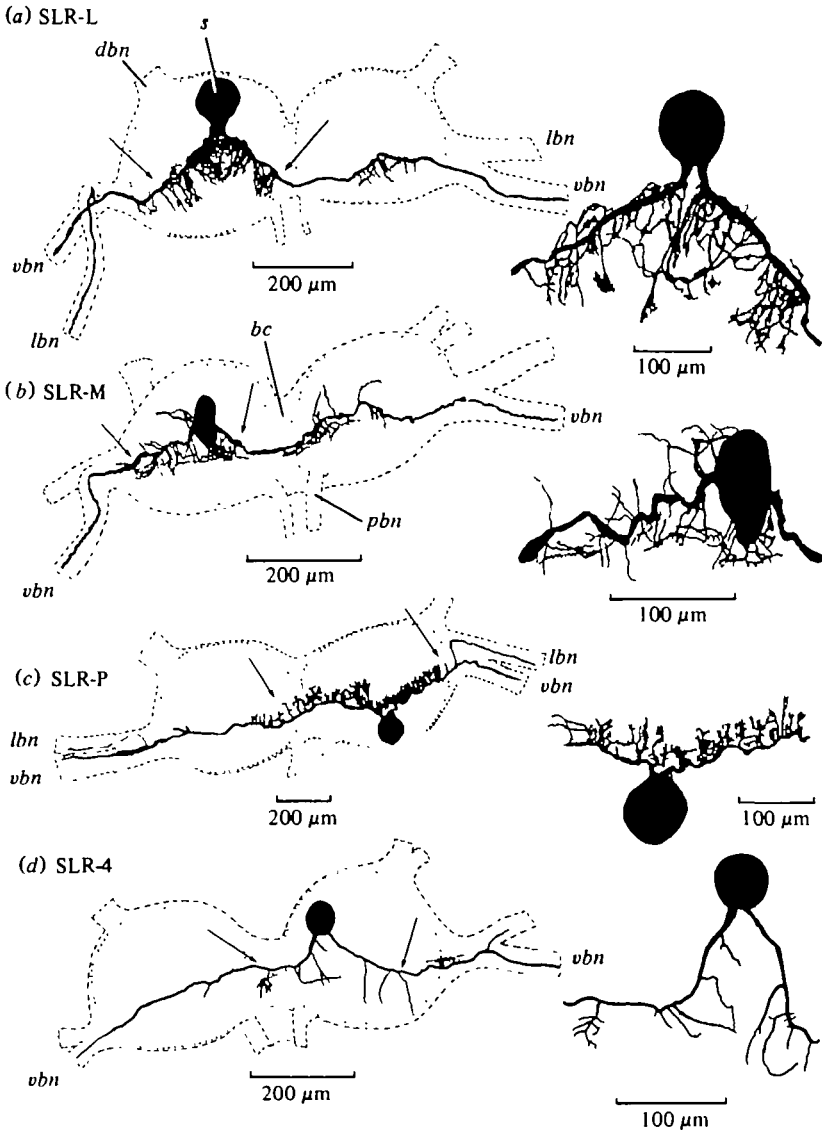


Fig. 5. Diagrams of typical examples of nickel-filled SLR neurone profiles of *Planorbarius* revealed by staining: (a) lateral SLR, (b) medial SLR, (c) phasic SLR and (d) 4th SLR. Profiles of the two members of any one pair of SLR neurones are similar. Ganglionic borders and the positions of some emergent nerves are indicated by dotted and dashed lines. Note that the profiles are not all drawn from exactly the same viewpoint. Diagrams to the right show, at higher magnification, those regions of the SLR neurones indicated by arrow heads on the drawings to the left. All cells have bilateral projections which, with the exception of SLR-L, are equivalent across the midline, and also show arborization within the ganglion contralateral to the soma, and within or adjacent to the buccal commissure. Abbreviations: *bc*, buccal commissure; *dbn*, dorsal buccal nerve; *lbn*, lateral buccal nerve; *pbn*, posterior buccal nerve; *s*, neurone soma, *vbn*, ventral buccal nerve.

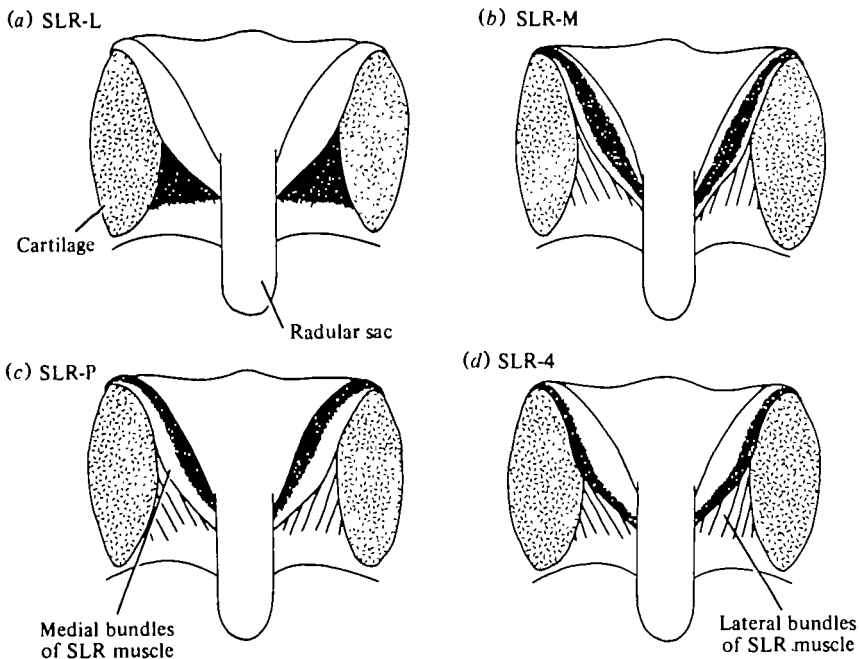


Fig. 6. Diagrams based on the dissection display of Fig. 1 to show the fields of innervation of the four SLR motoneurone types (pairs): (a) lateral SLR, (b) medial SLR, (c) phasic SLR, and (d) 4th SLR. Areas of innervation are heavily stippled; the two cartilage halves are stippled.

lateral muscles. The results of antidromic stimulation and nickel staining concurred and revealed that the peripheral axonal distribution of these neurones (Fig. 5) were confined to the lateral (*lbn*) and ventral (*vbn*) buccal routes, and that each of the four motoneurones within one ganglion was mirrored by that of its symmetrically placed partner across the midline. All the neurones were bipolar and, with the exception of the two SLR-Ls, had equivalent axonal pathways across the midline.

The dendritic ramifications of members of each pair of motoneurones were similar within and constant between animals. Arborization was most extensive in SLR-L (Fig. 5a), and minimal in SLR-4 (Fig. 5d), features which are consistent with the relative diversities of synaptic information which each of these neurone types receive (R. C. Brace & D. L. J. Quicke, in preparation). Prolific branching was principally confined to the initial segments of the two axons in all cells but some sparser branching occurred within the contralateral ganglion. Additionally there were fine branches originating from the axons as they traversed the buccal commissure.

Motor effects of identified SLR motoneurones

The spatial distributions of the motor responses evoked by intracellular stimulation of a member of each of the identified SLR motoneurone pairs are illustrated in Fig. 6. The ipsi- and contralateral responses produced by any one neurone were similar, and identical to those elicited by its contralateral partner, but distinct from those produced by activity in the other three pairs of cells.

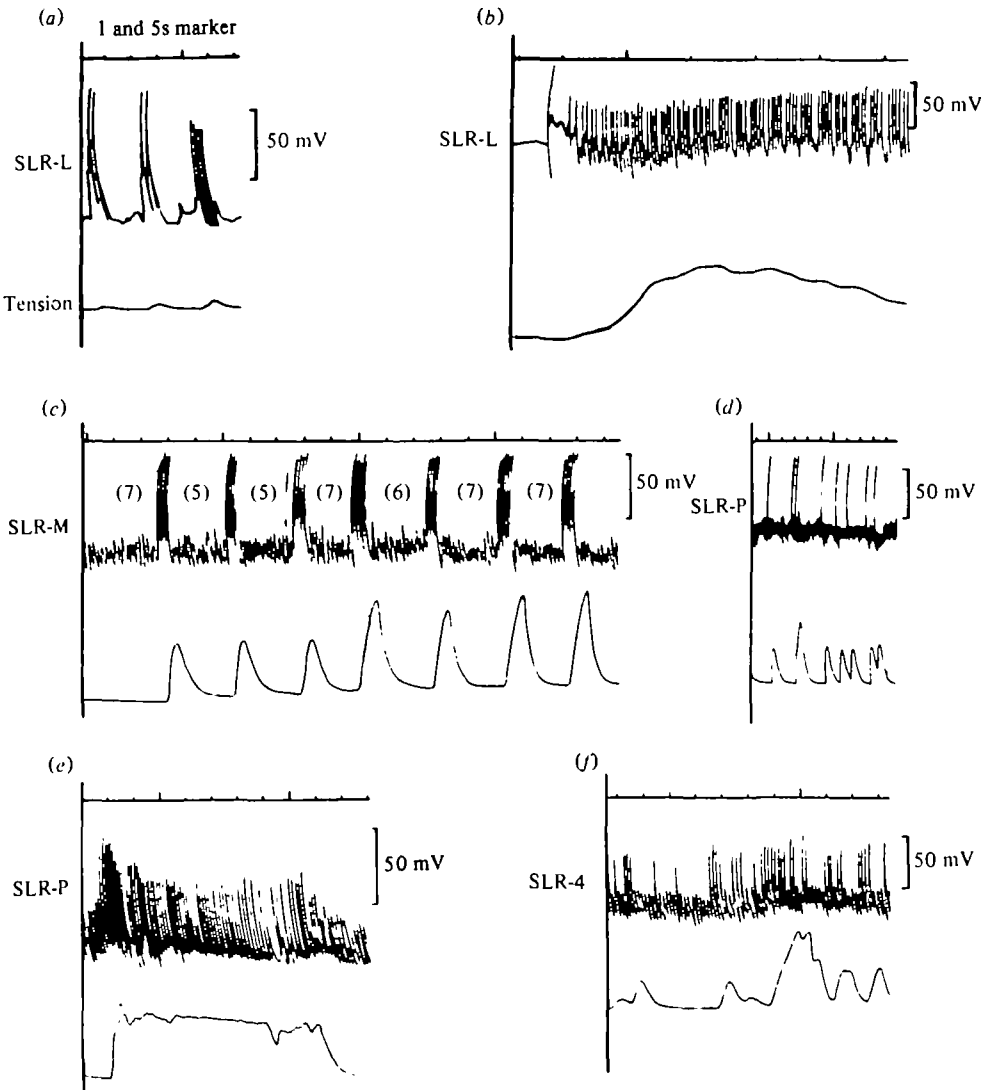


Fig. 7. SLR motoneurone activity and associated muscle responses in dissected buccal mass preparations (*a-b*). Evoked spiking in the ipsilateral SLR-L and muscle tension in lateral bundles of the SLR muscle. In (*b*) note that sustained spiking results finally in almost complete fatigue of the motor response. The slight neuronal accommodation occurring during this period was partly offset by steadily increasing the amount of depolarizing current injected (2–5 nA). (*c*) Bursts of spikes in an SLR-M motoneurone and responses in medial bundles of ipsilateral SLR muscle; each burst contains a similar number of spikes (in brackets). Note that there is potentiation of contraction during the initial bursts. (*d*) Spontaneous spiking in an SLR-P and responses from ipsilateral medial SLR bundles. (*e*) Prolonged evoked spiking in SLR-P produces little fatigue in the contralateral muscle. Note that accommodation of the neurone towards the end of this period is responsible for the slight lowering of tonus. (*f*) On-going spiking in SLR-4 and motor responses from the most lateral fibres of the medial region of the ipsilateral muscle. Here, and in Fig. 8, muscle tensions are not calibrated (see p. 118).

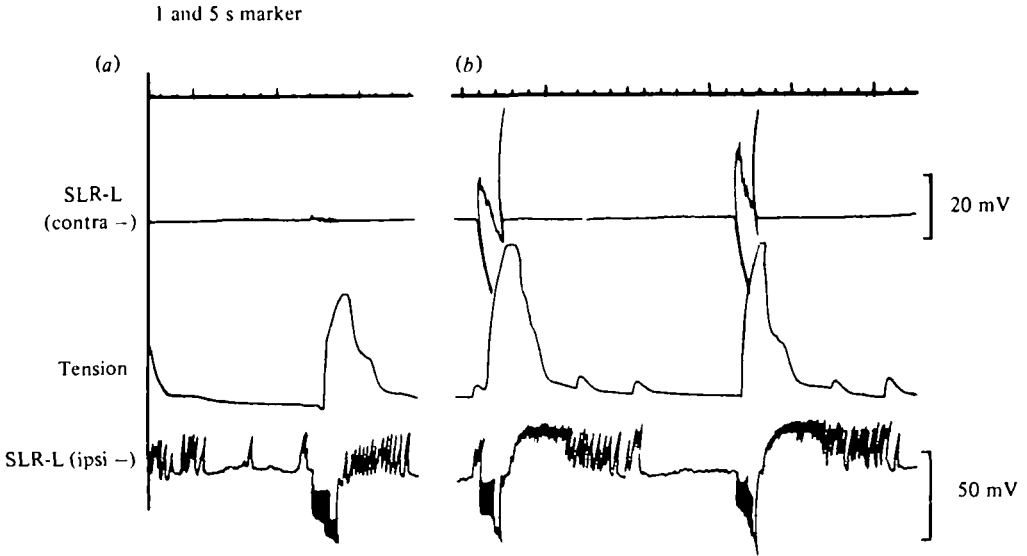


Fig. 8. To show summation of motor responses of an SLR muscle in a dissected buccal mass preparation, produced by activity in two SLR-L motoneurons. (a) Activity in the ipsilateral cell alone; note the electrically transmitted EPSP's in the partner SLR. (b) Both SLR-L's are depolarized resulting in a great enhancement of muscle contraction. After each contraction, the ipsilateral SLR-L receives a burst of EPSP's which gives rise to a small number of spikes which in turn causes two subsidiary muscle contractions. This feedback is relayed back to the motoneurons largely via interneuronal pathways (Brace & Quicke, in preparation).

SLR-L innervated the lateral areas of the muscles, SLR-M supplied the central longitudinal, dorsoventral slice of the medial regions and SLR-P innervated in particular the most medial bundles; the innervation of SLR-4 encompassed those medial fibres which fasten to the subradular epithelium. However, the populations of fibres innervated did not appear to be totally exclusive of one another; overlap was especially apparent between those of SLR-M and SLR-P. Some minor individual variation in the extents of these populations was noticed.

Single spikes in an SLR-L produced little or no motor response from the lateral bundles of fibres (Fig. 7a), but during a train of events individual responses facilitated and summated, though only a relatively slow increase in tension resulted (Fig. 7b); sustained stimulation quickly promoted fatigue. Less facilitation was necessary in the SLR-M neuromuscular units, and even short bursts of spikes in a motoneurone (Fig. 7c) evoked marked, but smooth contractions. In common with the SLR-L units, the magnitude of the tension increase elicited was closely tied to the number and frequency of action potentials in the motor cell, but fatigue was less evident. Activity in the SLR-M's caused, in almost intact buccal masses, posterior movements of the medial region of the radula, and of the radular sac. On the basis of behavioural observations and electrophysiological recordings from preparations displaying cyclical feeding movements (R. C. Brace & D. L. J. Quicke, in preparation), we conclude that their main functions are to tense the radula whilst it is applied to the substratum, and to help retract it soon after it is withdrawn.

Single spikes evoked in an SLR-P always elicited 'twitch-like' responses (Fig. 7d),

and prolonged depolarizations caused intense contractions. Rather unexpectedly, in view of the phasic motor responses, this unit proved resistant to fatigue during tetanus (Fig. 7*e*). In contrast to the largely unidirectional contraction resulting from depolarization of an SLR-M cell, activity in an SLR-P evoked a rolling movement of the innervated bundles of fibres. This vigorous action is characteristic of that occurring towards the end of radular retraction in each feeding cycle. Our electro-physiological recording suggests, however, that the SLR-P units may also be active during the rasping phase, and therefore can have two maxima of activity during each feeding cycle.

The motor responses of the SLR-4 units were similar to those of the SLR-L's (Fig. 7*f*), and thus the two types of unit embracing the lateral parts of the muscles which bring about lateral spreading of the radula during rasping, both have tonic properties.

Not unexpectedly, the simultaneous stimulation of both partners of any pair of neurones produced a summation of motor responses (Fig. 8).

When the SLR motoneurones were made to spike at physiologically normal frequencies, little neuronal accommodation, with the exception of the SLR-P's, was apparent (see Fig. 7). This exception parallels many arthropod examples in which phasically acting motoneurones (Burrows & Horridge, 1974; Atwood, 1976; Evoy, 1977) tend to accommodate more rapidly than do tonic units. Since the SLR neurones also displayed relatively little post-inhibitory rebound firing, it suggests that the motor responses are not to any large extent 'shaped' by intrinsic motoneuronal properties.

Electrical coupling between SLR motoneurones and with other cells

Despite systematically exploring all alternative pathways between the eight cells only one electrical connexion was consistently found. This linked the two SLR-L's (Fig. 9*a*). Spikes occurring in one cell evoked 1:1 short duration, excitatory post-synaptic potentials (EPSP's) in the other (Fig. 9*b*), with a constant latency of 2-3 ms. The coupling factor as recorded from the somata varied between 0.02 and 0.04; no directional rectification was noted. Also consistent with this synapse being electrical in nature, was the feature that despite hyperpolarization of the follower cell which altered the magnitude of PSP's caused by activity at chemical synapses, that of those conducted by the electrical synapse did not change accordingly.

At spike frequencies similar to (or greater than) those occurring during spontaneous activity, the resultant PSP's neither summed appreciably (Fig. 9*b*), nor took that cell markedly towards threshold. Fig. 9 (*c*, *d*) shows that PSP's produced in the partner cell may, nevertheless, sum with subthreshold chemical events to produce spikes. Thus we conclude that this synapse serves simply to aid synchronization of activity when one or both neurones are spiking. We feel that it is not unreasonable to suggest that this ephapse might reside within the buccal commissure where the two axons run together, and where each gives off fine branches which interdigitate closely. Similar apposition of corresponding retractor motoneurones occurs in *Lymnaea* (Goldschmeding, Bruins & Everts, 1977; Benjamin *et al.* 1979).

Berry (1972) described at length an electrical synapse linking SLR-L to a smaller, ipsilateral neurone (his cell 4), the position (\times) of which is depicted in Fig. 4. We have

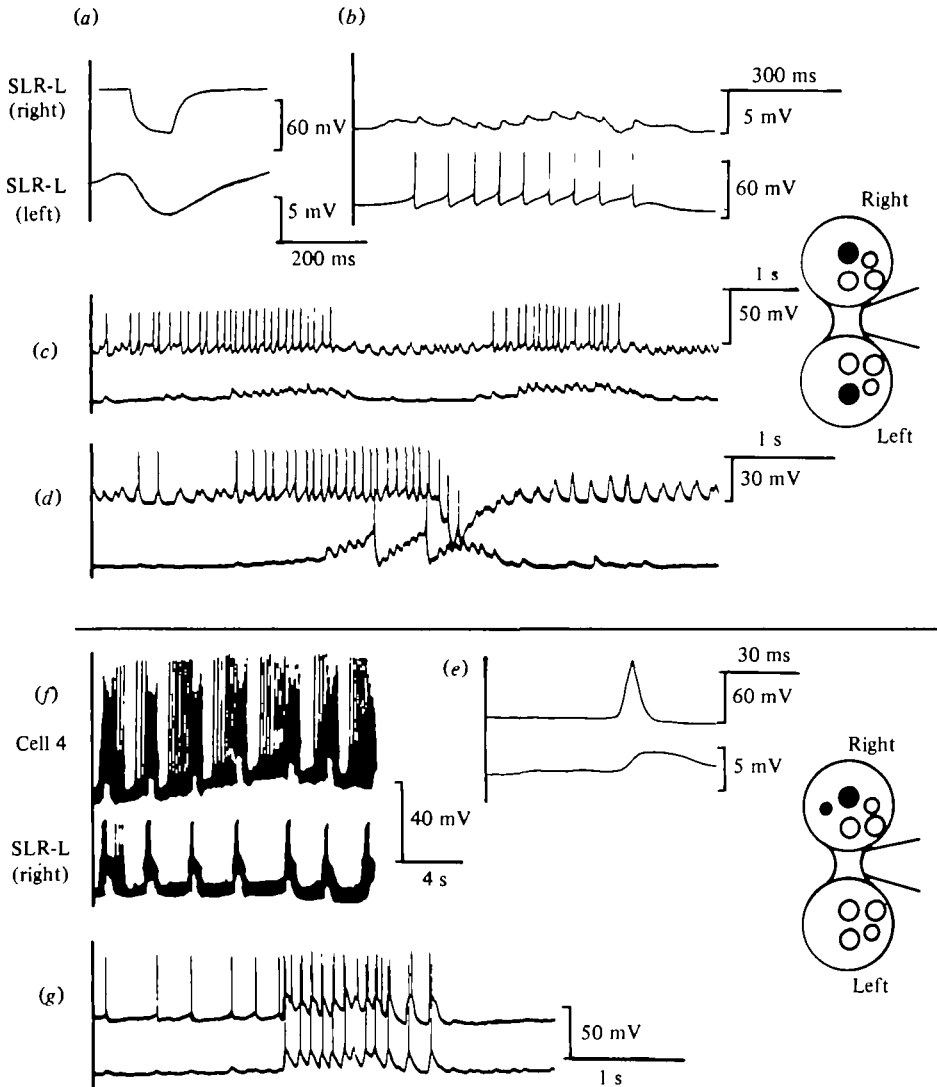


Fig. 9. (a-d) Electrical coupling between lateral SLR's (a) A hyperpolarizing pulse (2-3 nA) injected into one (right) produces an attenuated response in its partner. (b) Spikes evoked in one SLR (left) elicit electrotonically transmitted PSP's in the other: coupling ratio in (a) and (b) is 0.02-0.04. (c) SLR-L (right) is spiking owing to a more or less continuous barrage of chemical PSP's; its partner is receiving bursts of chemical PSP's. When the injection of depolarizing current into SLR-L (right) is timed (d) to occur during a PSP burst in the other neurone, the resultant PSP's sum with the chemical PSP's to produce two spikes. (e-g) Electrical coupling and activity of SLR-L (right) and ipsilateral cell (cell 4 of Berry (1972)). (e) A spike in cell 4 produces a short latency (2 ms) EPSP in SLR-L; coupling ratio is 0.03. (f) Burst activity in the same two neurones; note that cell 4 spikes for about 2 s before each intense burst of activity common to both neurones, and at this time (g) a train of discrete electrically transmitted PSP's is easily discerned in SLR-L. The diagrammatic inserts of the buccal ganglia show those cells whose activity is being monitored (filled circles), together with those of the remaining identified SLR motoneurons (unfilled circles).

been unable to determine the motor effect of this cell if any, though since it has an axon in an ipsilateral buccal route it seems likely that it might also be a motoneurone. The coupling factor of this second synapse was again small (0.05) (Fig. 9e). The two cells often exhibited in-phase bursting (Fig. 9f), though not all spikes occurring in the smaller cell were represented by ones in SLR-L. At these times, the former typically fired at a lower frequency for 2–4 s prior to each intense burst, and during these periods the resulting electrotonic EPSP's were easily discerned (Fig. 9g).

DISCUSSION

We have demonstrated that the innervation of the pair of supralateral radular retractor muscles of *Planorbarius* is complex, each muscle being supplied by at least eight motoneurones. This multiple innervation clearly affords behavioural flexibility to a motor system which we have shown to be multifunctional, participating in both radular rasping and retraction. Discussion of the organization of the SLR neuromuscular system can be conveniently divided into three topics which are considered in turn below.

Coupling between SLR motoneurones

The virtual absence of coupling between the synergistic motoneurones permits them to act independently of one another, thus allowing different combinations of motoneurones to be activated as required. In common with equivalent, non-coupled pools of motoneurones known in other gastropods (Lee & Liegeois, 1974; Siegler, Mpitsos & Davis, 1974; Kupfermann, Carew & Kandel, 1974; Mayeri *et al.* 1974), the selective distribution and phasing of an array of synaptic inputs (R. C. Brace & D. L. J. Quicke, in preparation) serves to bring about diverse patterns of spiking in the SLR population.

Neuromuscular units

We have shown that the specific division of the control of each muscle into four neuromuscular units is closely related to the differing mechanical demands met during the rasping and retractive phases of the feeding cycle. During the former, the muscles principally act as tensors, binding the radula to the odontophoral cartilage so as to oppose forces created at the radula-substratum interface. At this time, activity in the SLR-L and SLR-4 units spread and tense the radula, and reduce distortion of the cartilage. Evidence suggests that isometric contraction in the SLR-M and SLR-P units further reduces any tendency for the radula to travel forwards over the cartilage lip.

Radular retraction is signalled by a posterior retreat of the radula, brought about by activity in the SLR-M's, and then later by the SLR-P's; throughout this second phase, the more lateral parts of the muscles (SLR-L and SLR-4 units) are inactive and therefore relax, facilitating a folding of the radula which can then grasp gathered food items.

Surprisingly, the distinction between the rasping and retractive phases of the feeding cycle of a herbivorous gastropod has only been made recently within a neurophysiological context (Goldschmeding & de Vlieger, 1975; Goldschmeding, 1977; Benjamin & Rose, 1979; Rose & Benjamin, 1979), since it has been fully

Appreciated by functional morphologists for some time (Crampton, 1973; Hembrow, 1973). Such a division is significant from the neurophysiological point of view. Where a complex behaviour is split into two, as reported for the feeding cycle of *Helisoma* by Kater (1974) (protraction and retraction), the underlying interneuronal circuitry need consist of no more than interneurons which excite one group of motoneurons whilst inhibiting antagonists. However, when three or more phases are involved, control must be more complex, incorporating the sequential activation of motoneurons as appears to be the case in *Tritonia* (Bulloch & Dorsett, 1979a, b).

Allied with the different functions performed by the various neuromuscular units we have found diversity in their responses. The SLR-L and SLR-4 units have tonic properties which are particularly suited to their role in exerting a steady build-up of muscle tonus to accurately counteract resistance met by the radula during rasping. In contrast, the phasic responses of the SLR-P's are especially suited to providing the rapid withdrawal of the radula during retraction which ensures that food gathered by the radula is subsequently retained. The SLR-M's play a major part in both feeding phases, and correspondingly display intermediate characteristics. Individual neuromuscular systems which are either 'fast' or 'slow' have already been described in gastropods (Kater, Heyer & Hegman, 1971; Heyer, Kater & Karlsson, 1973; Orkland & Orkland, 1975; Cohen *et al.* 1978), but the SLR system of *Planorbarius* is the first example in which it has been demonstrated that the two types of response may be exhibited by a single muscle.

A comparison of the retractor system of *Planorbarius* with that of the radular closers of *Aplysia* (Cohen *et al.* 1978) shows that the latter has far fewer motoneurons, each muscle being supplied by only two ipsilateral cells both of which, however, innervate all fibres. These discrepancies can easily be related to the dichotomy in behaviour of the muscles. Unlike the SLR's, the closers are responsible for only one, fairly stereotyped, grasping action of the radular halves, and therefore the emphasis is on the fine control of overlapping populations of muscle fibres, rather than on the interplay of a larger number of specialized units. To this end, peripheral integration (pre- and postsynaptic) is important in *Aplysia* in determining the final motor output; the muscle fibres are multi-terminally innervated and do not support action potentials. Unfortunately we know little about peripheral events in *Planorbarius* to ascertain whether similar integration occurs. Indeed it is not yet known whether within each neuromuscular unit, all fibres are polyneuronally innervated by partner SLR's or alternatively whether there are two sub-populations of fibres, each singly innervated.

Bilateral motoneuronal innervation

A cursory consideration of the bilateral innervation provided by all of the SLR motoneurons suggests that it is superfluous for interneuronal circuitry (R. C. Brace & D. L. J. Quicke, in preparation; see also Benjamin & Rose (1979) for *Lymnaea*) can exert a high degree of synchrony across the midline. There is, however, one feature of bilateral coordination at the motoneuronal level which cannot be matched by corresponding interneuronal projections. Equal output from the two muscles is ensured, irrespective of any discrepancies in spiking between bilaterally homologous motoneurons. Since discrepancies of this nature are always likely to occur owing to either differential lability of transmission across the chemical synapses which link the

interneurons to the motoneurons, or to slight inequalities in intrinsic motoneuronal characteristics, this novel property can be of consequence. That strict regulation of activity across the midline in the radular system of *Planorbarius* is of especial importance is borne out by the construction of the radular assembly in which the radular membrane exerts strong mechanical linkage between the two sides.

Therefore, in behavioural terms, we conclude that the SLR motor system caters solely for strictly anteroposteriorly directed excursions of the radular over the cartilage. Skewing movements which could easily lead to a marked lowering of mechanical efficiency, are not tolerated.

The mechanisms of bilateral coordination of locomotory behaviours have been investigated in some detail in insects (Pearson, Fournier & Wong, 1973; Burrows, 1975*a*) and annelids (Ort, Kristan & Stent, 1974). It appears to be overwhelmingly achieved by either sets of interneurons distributing information in a manner analogous to the motoneuronal supply of *Planorbarius*, or by coupling between groups of interneurons on either side of the midline. The axonal projections of the motoneurons are predominantly, unilaterally distributed (Evoy, 1977; Fournier & Pearson, 1977), though there is one reported example of bilateral motoneuronal innervation (Miller, 1973; Burrows, 1975*b*). Significantly, this exception concerns the innervation of spiracles in the locust (and other insects). These are structures which are, in common with the retractor muscles, mechanically coupled across the midline by, in this case, air in the tracheal trunks. Presumably strict bilateral control of their activity is particularly necessary during ventilatory pumping.

So far we have not taken into consideration the possibility of any dissimilarity of events occurring between the neuromuscular junctions on the two retractor muscles of *Planorbarius* which, of course, would detract from the overall strategy of bilateral motor equivalence. Visual and preliminary electrophysiological observations suggest, however, that little discrepancy of this nature occurs, though admittedly we have not yet explored possible unilateral spike failure using a wide range of motoneuronal spiking frequencies.

On the basis of the morphology of identified buccal retractor motoneurons, it seems likely that the motoneuronal arrangement revealed here is not unique to *Planorbarius*; it apparently also occurs in *Lymnaea* (Goldschmeding *et al.* 1977; Benjamin *et al.* 1979), and in the opisthobranchs, *Pleurobranchaea* (Siegler *et al.* 1974; Siegler, 1977) and *Navanax* (Woollacott 1974). Its role in the last is presumably to ensure symmetrical dilations and contractions of the wall of the buccal mass, the radula having been lost.

Integration of the activity of the SLR neuromuscular units of *Planorbarius* is reinforced by sensory feedback arising from receptors which are excited by contractions of the retractor muscles. Information derived from this source is relayed back to the SLR motoneurons largely via interneurons (see Fig. 10). Furthermore, this activity does not provide solely for compensatory reflexes for feedback may be inhibitory as well as excitatory. Importantly, the pattern of excitation and inhibition distributed to the array of motoneurons is dependent upon the patterns of on-going synaptic input arriving at the various cells and these, in turn, can be related to the various functional roles 'demanded' from the muscles at different times during the feeding cycle (R. C. Brace & D. L. J. Quicke, in preparation). Nevertheless an

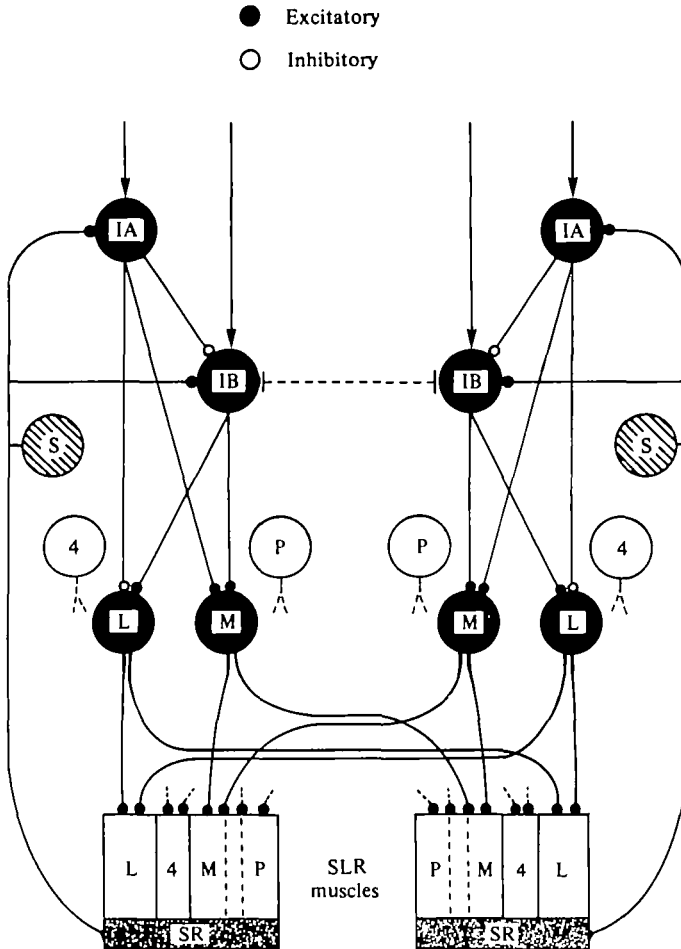


Fig. 10. A simple model for some components of the SLR control system. The four types of neuromuscular unit (L, lateral; M, medial; P, phasic; 4, 4th) are shown, together with sensory neurones (S) which mediate feedback elicited by contractions of the SLR muscles, and two pairs of interneurons (IA, IB) which collectively are capable, upon a change of higher order inputs, of imposing a feed-forward substitution of their inputs on to the array of motoneurons. Note that each motoneurone innervates only a limited region of each muscle but that there is apparently some overlap of the fields of innervation of SLR-M and SLR-P. Complete circuitry is shown only for the SLR-L and SLR-M units. A change in higher order input can switch commands between IA and IB, and this produces equivalent substitution of the SLR-M (excitation), but non-equivalent substitution (excitation-inhibition) to SLR-L. Modifications of this type may be responsible for transitions between the discrete activity regimes exhibited at different stages of the feeding cycle and are described and discussed at length in Brace and Quicke (in preparation). The dotted line linking the IB neurone groups indicates the synchronization of interneuronal activity across the midline.

alterations in spiking in partner SLRs brought about by unilaterally directed feedback from either muscle will always result in bilaterally equivalent modifications to the SLR motor output owing to the constraints imposed by the dual-fold bilateral motoneurone projections.

There is one final point that deserves mention. As each SLR-L has an asymmetrical,

bilateral axonal distribution, there must presumably be at least some minor difference between the motor responses it evokes in the two muscles. It is therefore interesting that the members of this pair of motoneurons were the only ones found to be electrically coupled. We therefore infer that the function of the synapse is to act as a fail-safe, guarding against minor discrepancies in activity of the partner SLR's which, *in this instance*, would bring about non-equivalent motor responses from the two muscles. Exactly why these neurons have this type of axonal distribution is not understood at present.

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